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**THE DISTRIBUTION OF MERCURY
WITHIN THE TISSUES
OF FRESHWATER FISH**

1975



Ontario

Ministry
of the
Environment

The Honourable
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Deputy Minister

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THE DISTRIBUTION OF MERCURY WITHIN THE
TISSUES OF FRESHWATER FISH

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INORGANIC TRACE CONTAMINANTS SECTION
ONTARIO MINISTRY OF THE ENVIRONMENT
AUGUST, 1975

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INTRODUCTION

The distribution of mercury within the tissues of fish has been studied from the point of view of sampling and chemical analysis by various workers (1, 2) but most previous studies of this nature have been directed towards elucidating the uptake and elimination mechanisms in fish (3 - 6).

The original purpose of this study was to compare fish muscle sampling techniques for general mercury monitoring purposes. The method used by our laboratory was to fillet the fish, homogenize the fillets, and sample the homogenate. Other laboratories have used the analysis of a "snip" sample of flesh to estimate the average mercury concentration. The sub-sampling of fillets, both longitudinally and axially, afforded us the opportunity to assess whether there are significant variations in muscle mercury concentration throughout the fish muscle, by characterizing each muscle sub-sample by its lipid and water content. The organs and other parts of the fish were also analyzed to obtain an overall distribution of mercury throughout the fish.

II: EXPERIMENTAL

A: SAMPLING:

This report is based on findings from 6 individual fish, representing 5 species. Each fish was sub-sampled and analyzed many times (up to 88 muscle analyses per fish) to establish

the mercury, water and lipid content of muscles, skin, internal organs, and bones. The six fish were:

- 1) Channel Catfish: Two individuals, both taken from Lake St. Clair in Southern Ontario (see Figure 1). Both were female; one was 44 cm. long and weighed 1228 gm. the other was 60.3 cm. long and weighed 2950 gm.
- 2) Carp: One female taken from Lake St. Clair, with a length of 75 cm. and a weight of 10,130 gm.
- 3) Walleye: One female, taken from Lake St. Clair near Tremblay Creek. This fish weighed 2380 gm. and was 60 cm. long.
- 4) Northern Pike: A female, taken from Moira Lake in eastern Ontario (see Figure 1). This fish was 87 cm. long and weighed 4662 gm.
- 5) Lake Trout: A male, taken from Lake Timagami in northern Ontario. This was a large fish, 100 cm. long, and weighed 12,485 gm.

These fish were gill-netted, frozen, and submitted for analysis. They were allowed to thaw in the laboratory, where they were sectioned and sampled as shown in Figure 2. The same general procedure was followed for all samples. After longitudinal sectioning, each fish was further sub-divided as shown in Figure 3. These samples were used to obtain estimates of

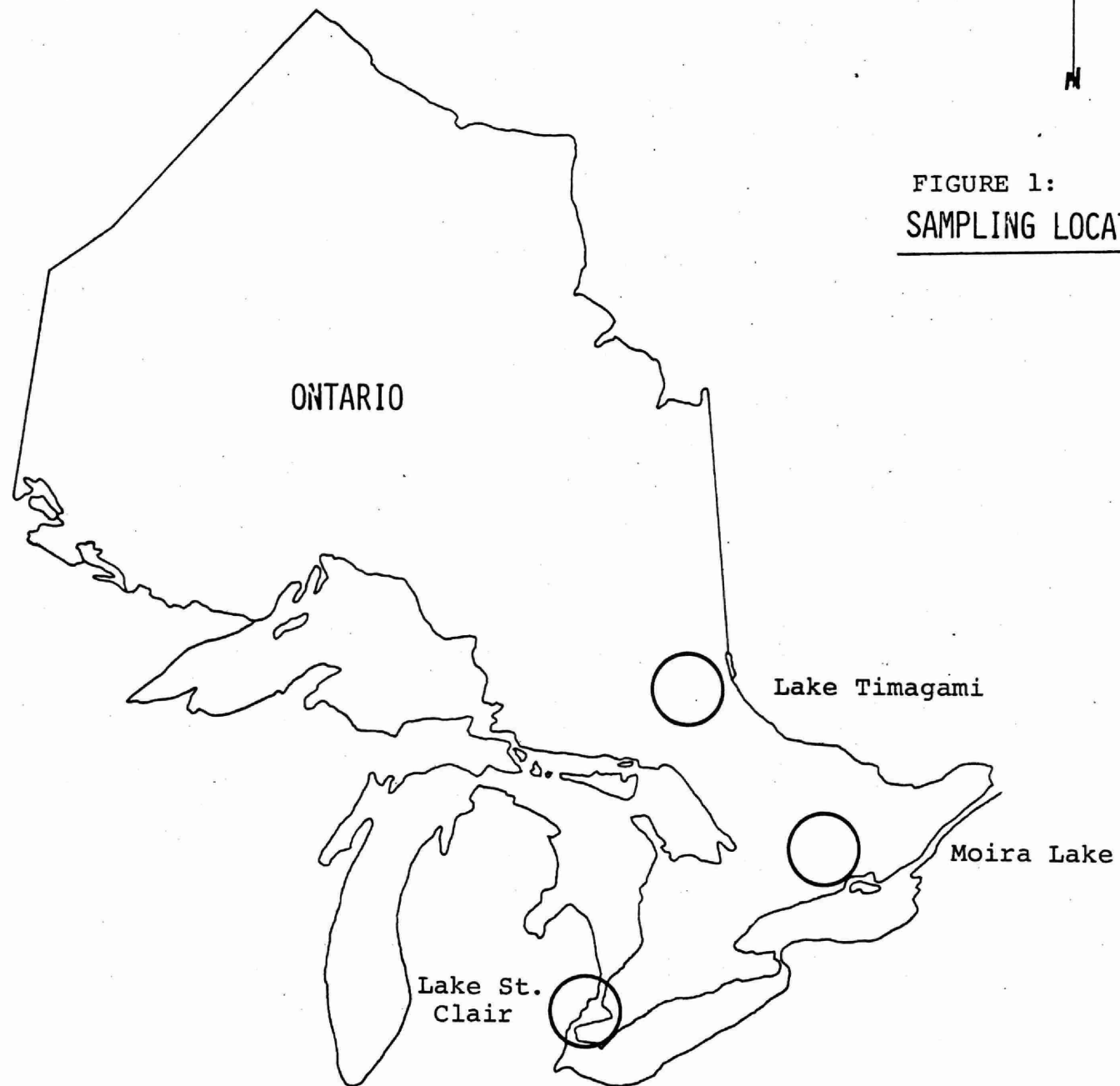
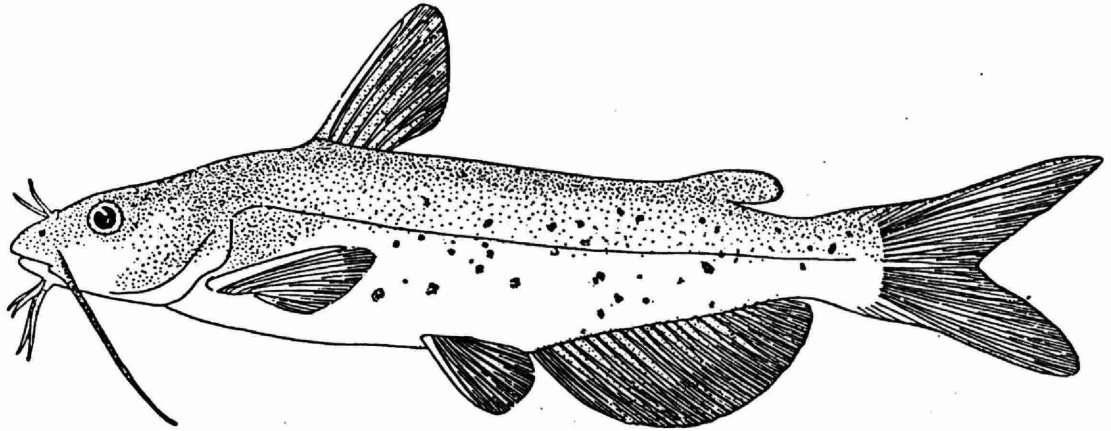
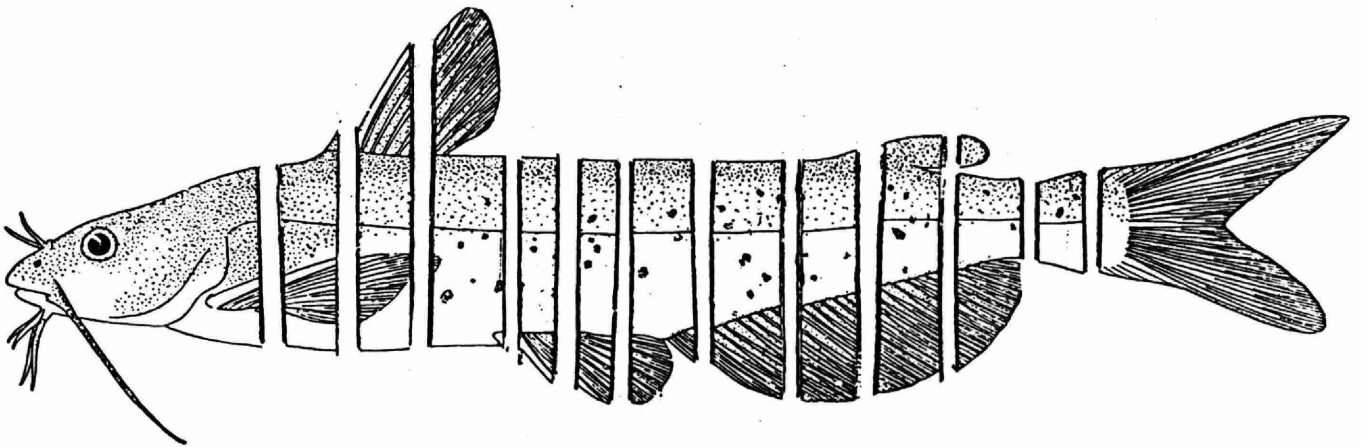


FIGURE 1:
SAMPLING LOCATIONS

FIGURE 2:



LONGITUDINAL SECTIONING OF CATFISH



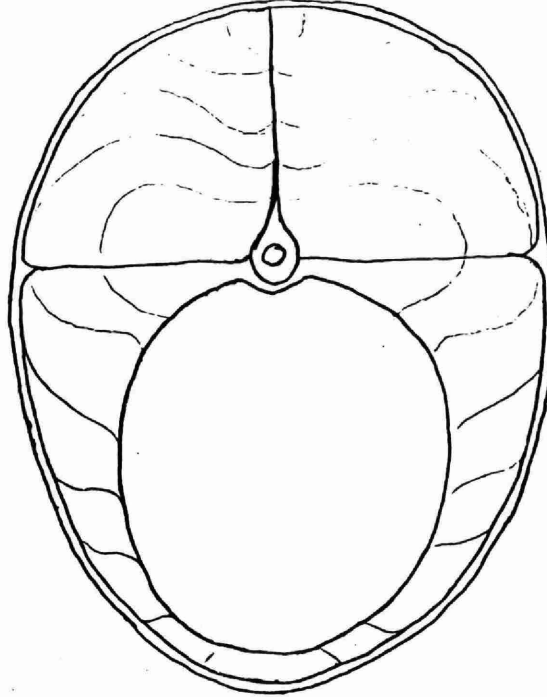
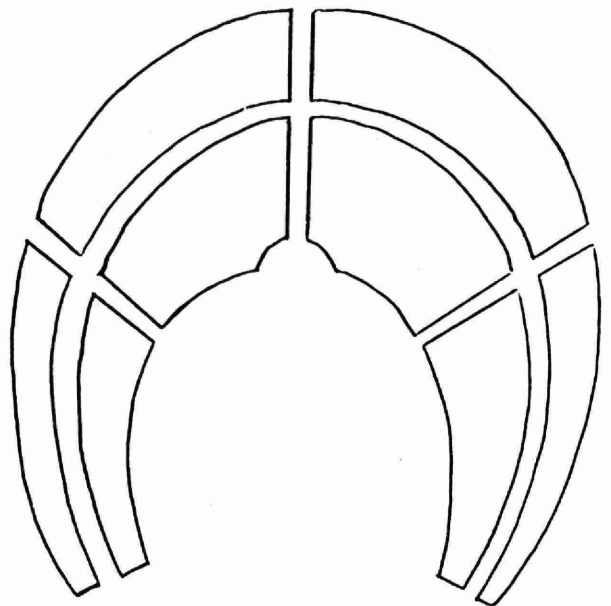
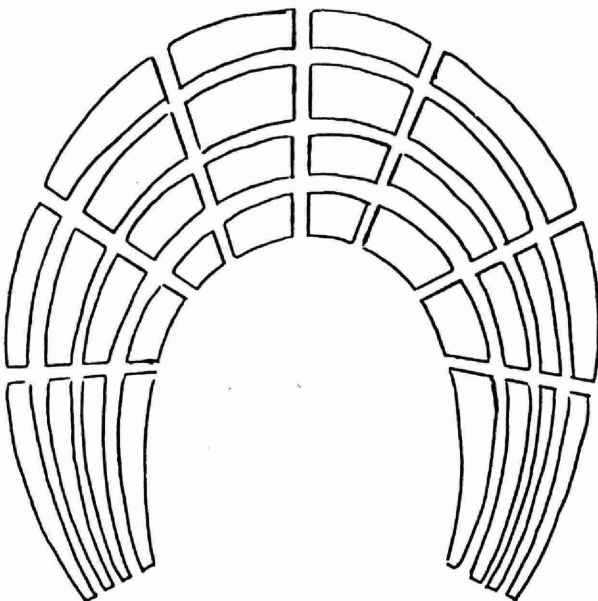


FIGURE 3:



SUBSAMPLING OF CROSS SECTIONS

the concentration of mercury in each section of muscle, and they allowed an examination of variations from front to back, and from internal to external muscle, for each fish.

The fish were also dissected, and the following organs removed for analysis: heart, kidney, liver, brain, intestines, intestine contents, gonads, skin. Some fish were further dissected to yield eyes, lenses, scales, bone and gills.

B: ANALYTICAL:

The samples were analyzed for total mercury by the Hot Block digestion, followed by flameless atomic adsorption spectrophotometry (7). Methyl mercury was determined on some samples, by a modified version of Westoo's technique (7, 8).

Lipid and water content of the muscle portions were determined by azeotropic distillation with benzene (9).

C: STATISTICAL:

The statistical results used in this study were normalized where necessary to facilitate comparisons between fish. All relationships were examined by the use of product moment correlation coefficients (10).

Regression lines were calculated by the method of least squares, and slopes and correlation coefficients were tested for signi-

ficance with t statistics. All calculations were performed on a Hewlett-Packard 9830A programmable calculator according to standard formulae.

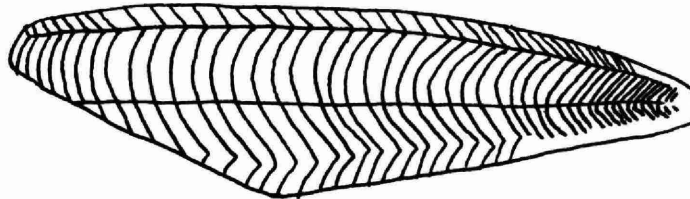
III: RESULTS AND DISCUSSION

A: VARIATION IN MERCURY CONCENTRATION ALONG THE LENGTH OF FISH:

Studies by Westoo (1) and earlier studies in our laboratory indicated that the variation in the concentration of mercury along the length of a fish was typically about the same as the variation due to analytical methodology. Although no statistical significance can be attributed, it was observed that the mercury concentration in muscle taken from the tail end of the fish was invariably lower than the concentration in muscle from the head of the fish. This observation can be rationalized in view of the increased proportion of connective tissue (myocommata) relative to muscle tissue.

Figure 4 shows a fillet of fish with myocommata, or connective tissue between the muscle tissue blocks. The relative proportion of myocommata to muscle corresponds inversely with the pattern observed for mercury - they increase in density towards the tail.

FIGURE 4



MYOCOMMATA IN FISH FILLET

Assuming that mercury is chemically bound to fish protein, one would expect the mercury concentration to be higher near the head of the fish, where there is a greater abundance of muscle cells proper, than near the tail. This effect of increasing concentrations of myocommata and diminishing mercury values, is even stronger with older fish, and our study used quite mature individuals.

B: VARIATION IN MERCURY CONCENTRATION THROUGH CROSS-SECTIONS OF FISH:

Table 1 shows the variation in mercury concentration from the exterior to interior muscle sections of the fish. Both catfish and the carp show significantly higher mercury and water in the interior muscle portions, while the lipid concentration is greater in the exterior portion. There is no significant difference in the results for the other species.

TABLE 1 :

MERCURY CONCENTRATION: VARIATION IN FISH MUSCLE

Species	Muscle Location	Hg (ppm)	% Lipid	% Water
Northern Pike	Exterior	2.18	3.42	74.69
	Interior	2.19	3.92	74.45
Walleye	Exterior	2.21	7.21	66.41
	Interior	2.27	8.32	64.50
Lake Trout	Exterior	1.04	15.55	56.36
	Interior	1.05	16.82	58.02
Carp	Exterior	0.46	24.61	53.65
	Interior	0.57	13.89	60.38
Catfish (2950)	Exterior	1.43	17.94	59.15
	Interior	1.55	12.59	63.22
Catfish (1228)	Exterior	0.76	18.66	55.71
	Middle	1.01	11.21	63.84
	Interior	1.14	4.49	71.17

C: RELATIONSHIPS BETWEEN MERCURY, WATER, AND LIPID CONCENTRATIONS:

a) The inter-relationships between these three muscle constituents are shown in Table 2. For all the fish, a strong negative correlation between the water and lipid content of the muscle portions was observed. This agrees well with previous reports (11), and these relations are shown graphically in Figures 5-7. The sum of these two constituents remained approximately constant for most of the species.

b) Figures 8-10 show the relations between mercury and lipid concentrations in the muscle portions for all six fish. The mercury concentration scales have been normalized to the average muscle mercury concentration to facilitate comparison. It can be seen that there are significant negative relationships between the lipid content and mercury concentration for both catfish and carp. Also, there were corresponding significant positive relations between the water content of the muscle and its mercury concentration for these species. The pike, walleye, and lake trout showed no significant relationships.

c) Figures 11 and 12 show the high degree of reproducibility between the two catfish used in the study. The slopes of the two mercury-lipid lines are virtually the same, but the larger catfish had a higher average lipid concentration.

TABLE 2:

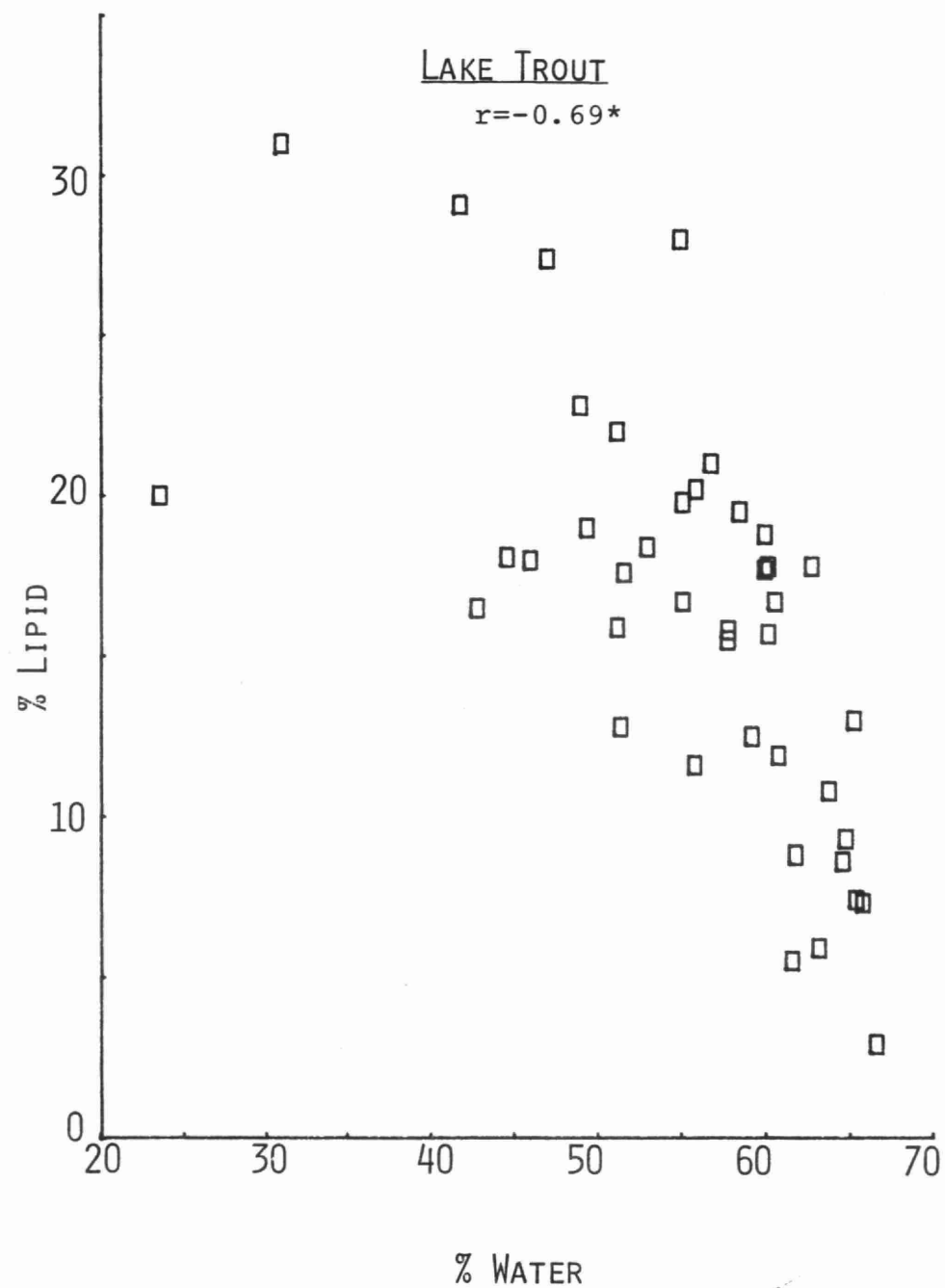
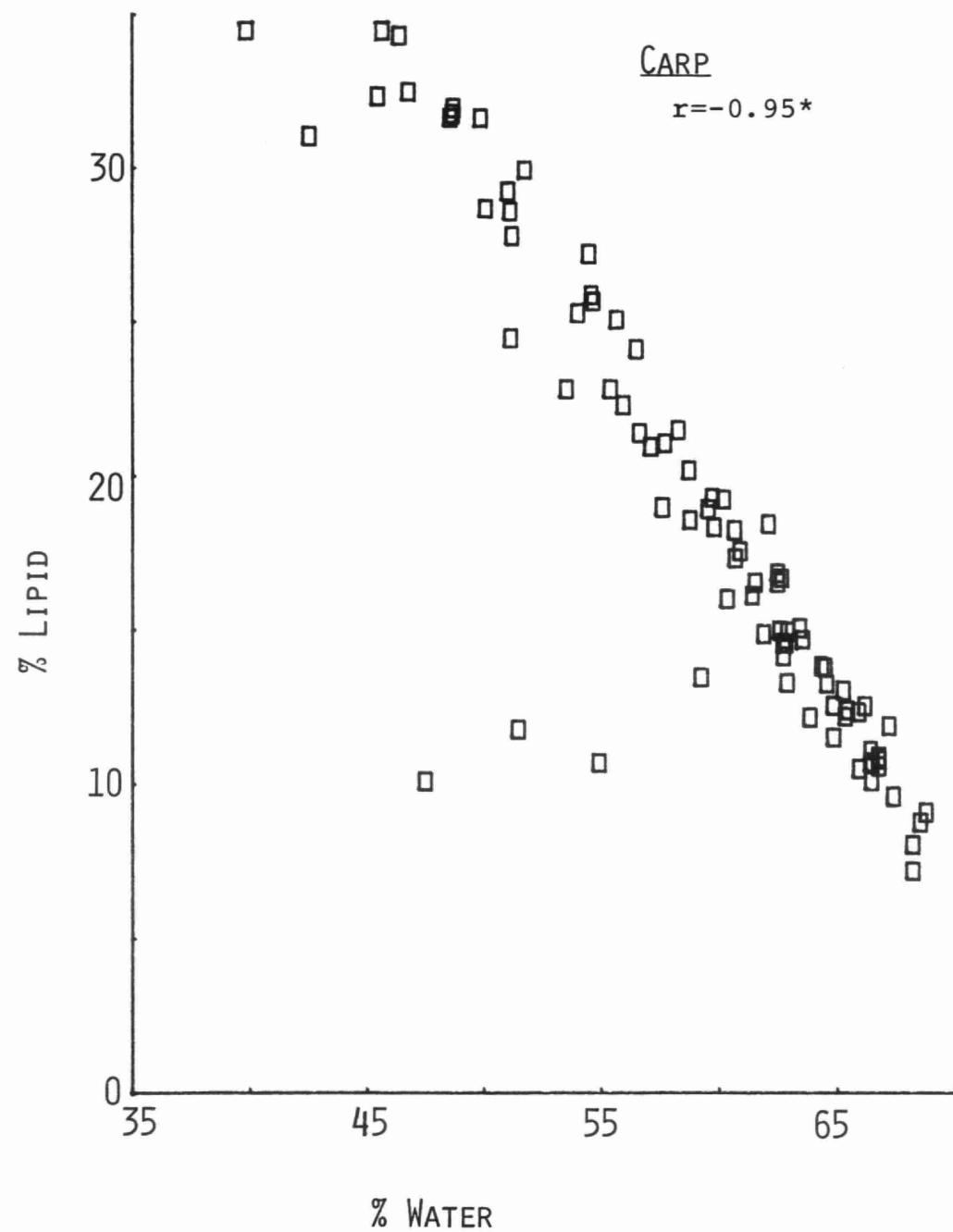
CORRELATION MATRICES

PIKE				WALLEYE			
	Mercury	Lipid	Water		Mercury	Lipid	Water
Mercury	—	-0.20	0.02	Mercury	—	0.28	-0.46*
Lipid		—	-0.83**	Lipid		—	-0.81**
Water			—	Water			—
CATFISH I				CATFISH II			
	Mercury	Lipid	Water		Mercury	Lipid	Water
Mercury	—	-0.72**	0.67**	Mercury	—	-0.66**	0.63**
Lipid		—	-0.89**	Lipid		—	-0.90**
Water			—	Water			—
CARP				LAKE TROUT			
	Mercury	Lipid	Water		Mercury	Lipid	Water
Mercury	—	-0.61**	0.55**	Mercury	—	-0.21	0.05
Lipid		—	-0.95**	Lipid		—	-0.69**
Water			—	Water			—

* significantly different from zero ($P < 0.05$)

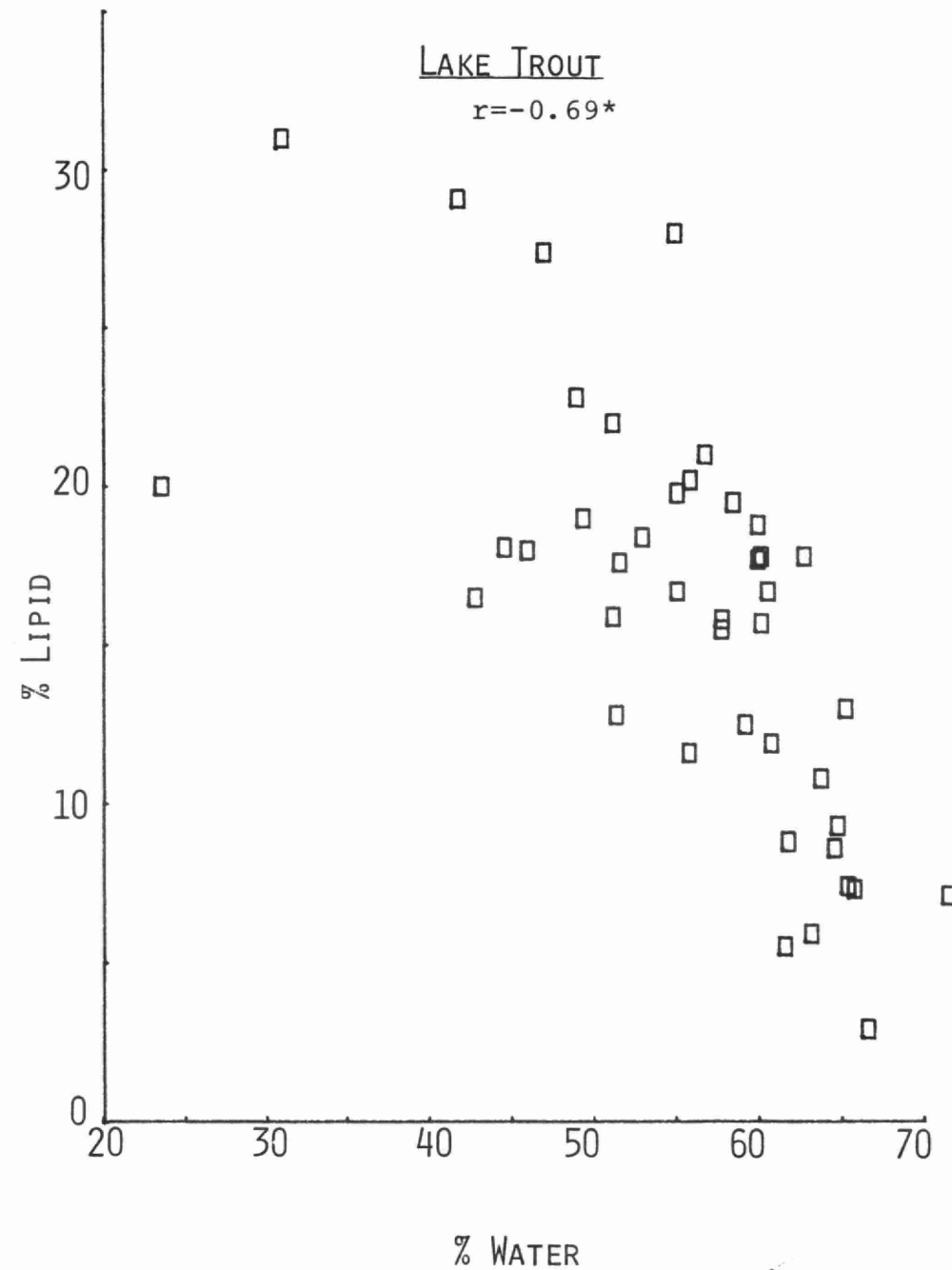
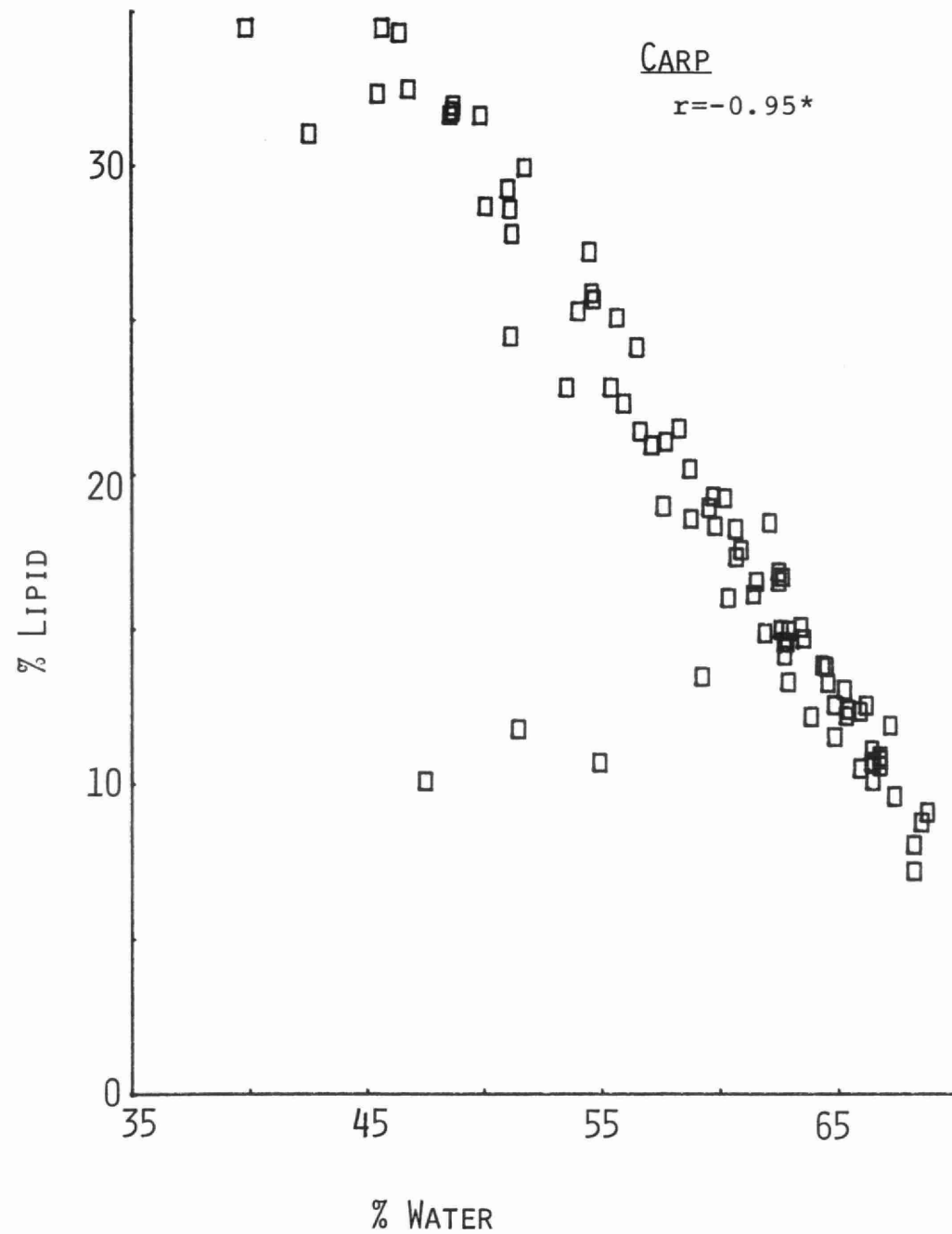
** significantly different from zero ($P < 0.01$)

FIGURE 5: RELATIONSHIP BETWEEN LIPID AND WATER CONTENT



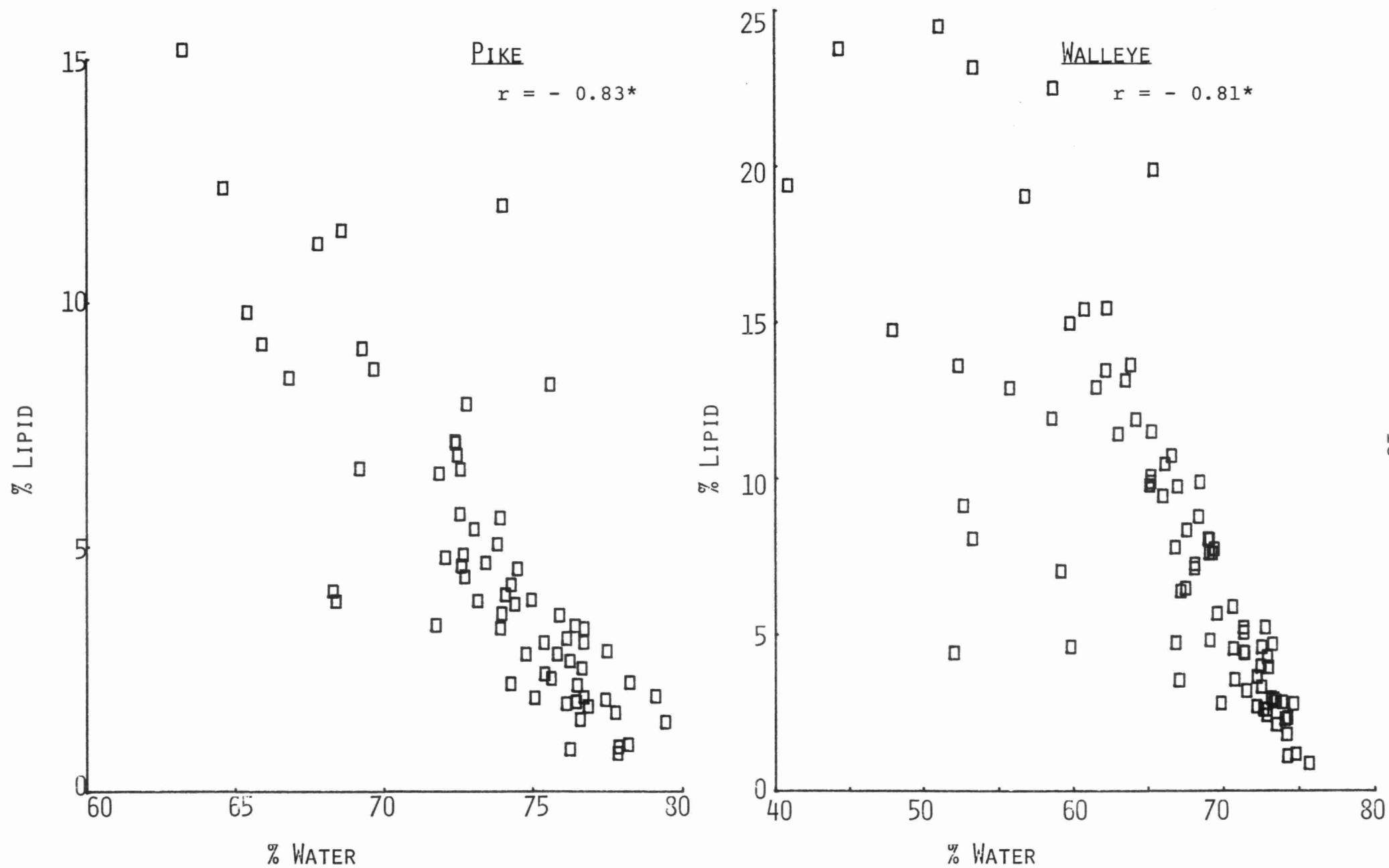
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FIGURE 5: RELATIONSHIP BETWEEN LIPID AND WATER CONTENT



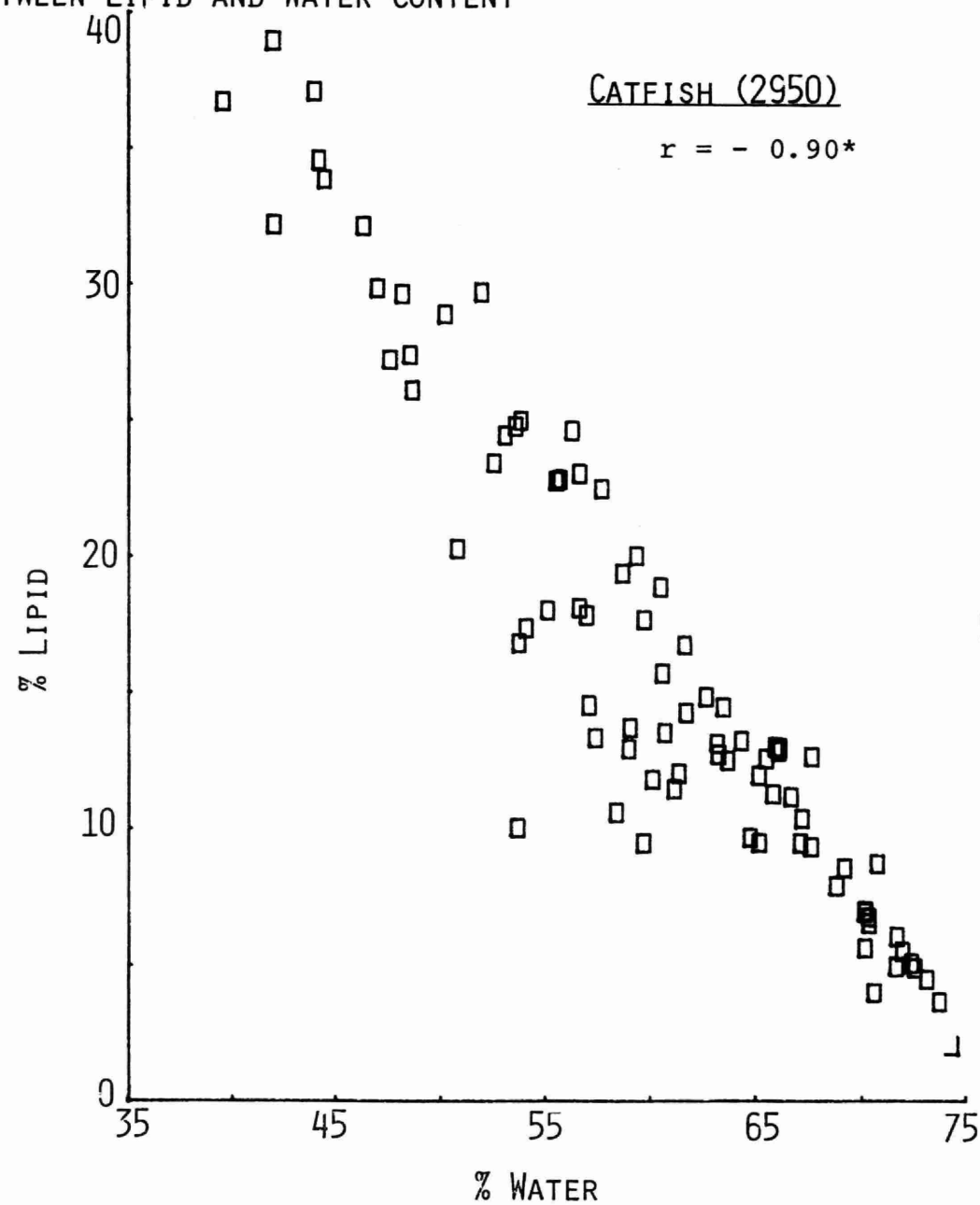
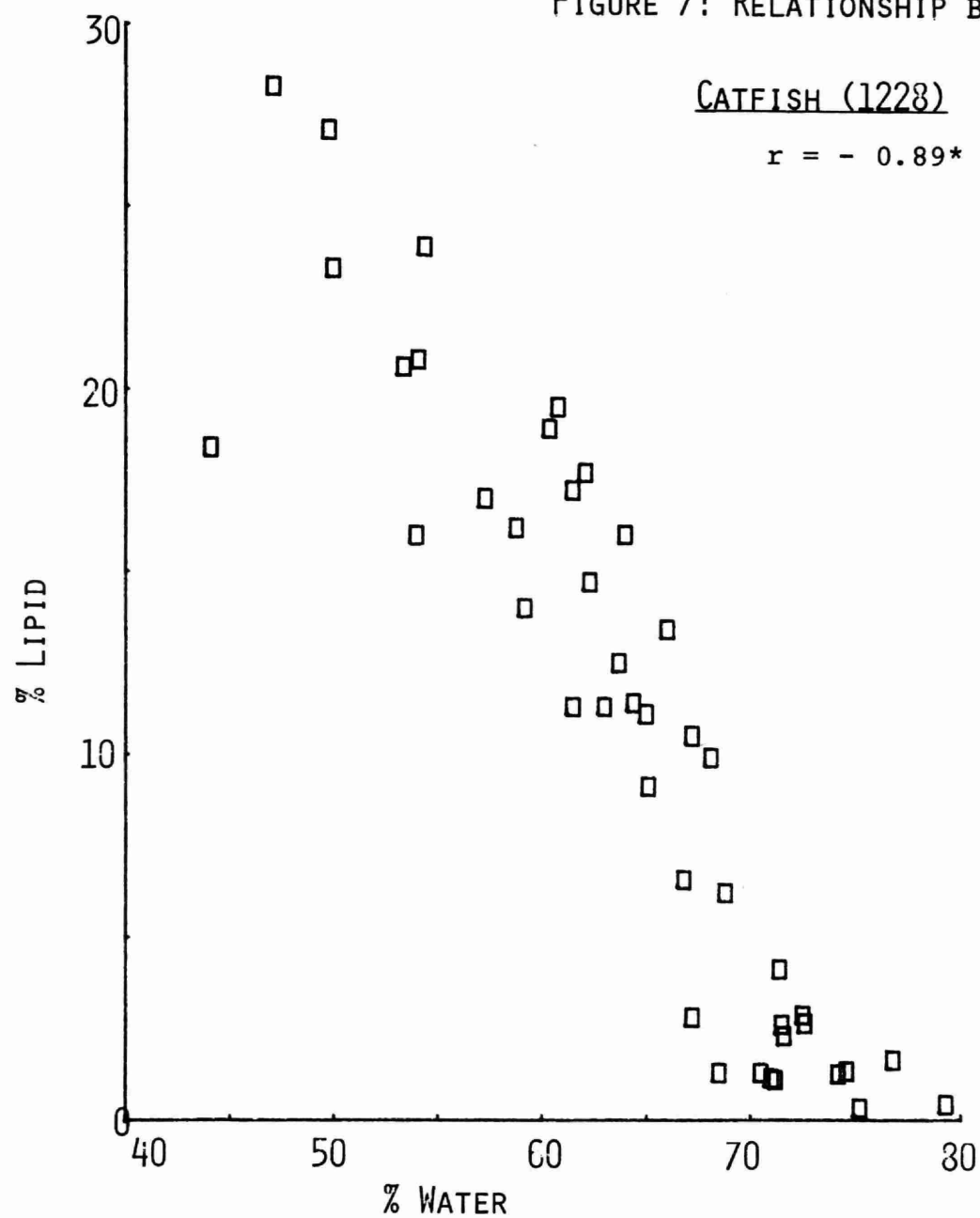
* SIGNIFICANTLY DIFFERENT FROM ZERO ($P < 0.01$)

FIGURE 6: RELATIONSHIP BETWEEN LIPID AND WATER CONTENT



* SIGNIFICANTLY DIFFERENT FROM ZERO ($P < 0.01$)

FIGURE 7: RELATIONSHIP BETWEEN LIPID AND WATER CONTENT



* SIGNIFICANTLY DIFFERENT FROM ZERP ($P < 0.01$)

FIGURE 8: RELATIONSHIP BETWEEN NORMALIZED MERCURY CONCENTRATION AND LIPID CONTENT

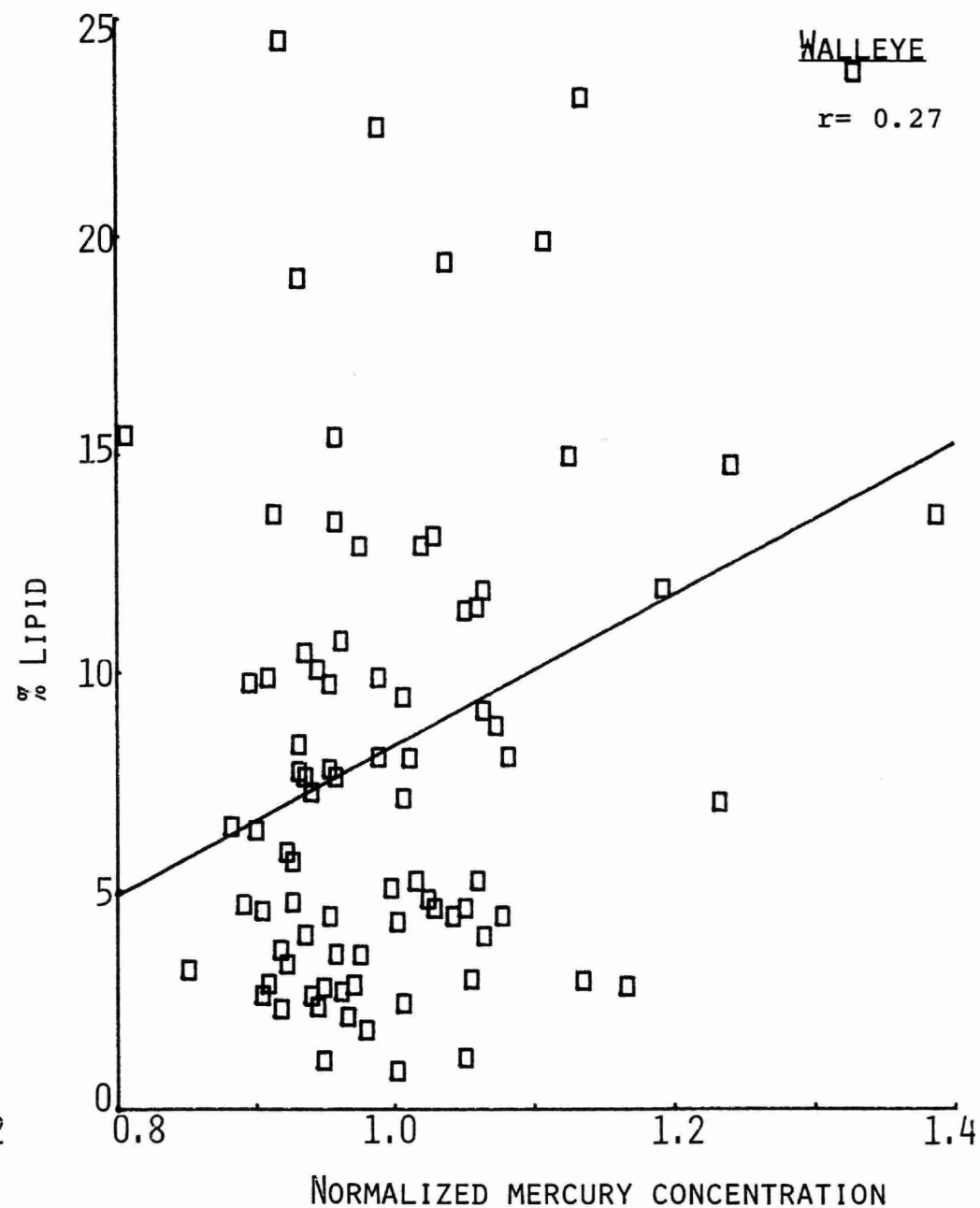
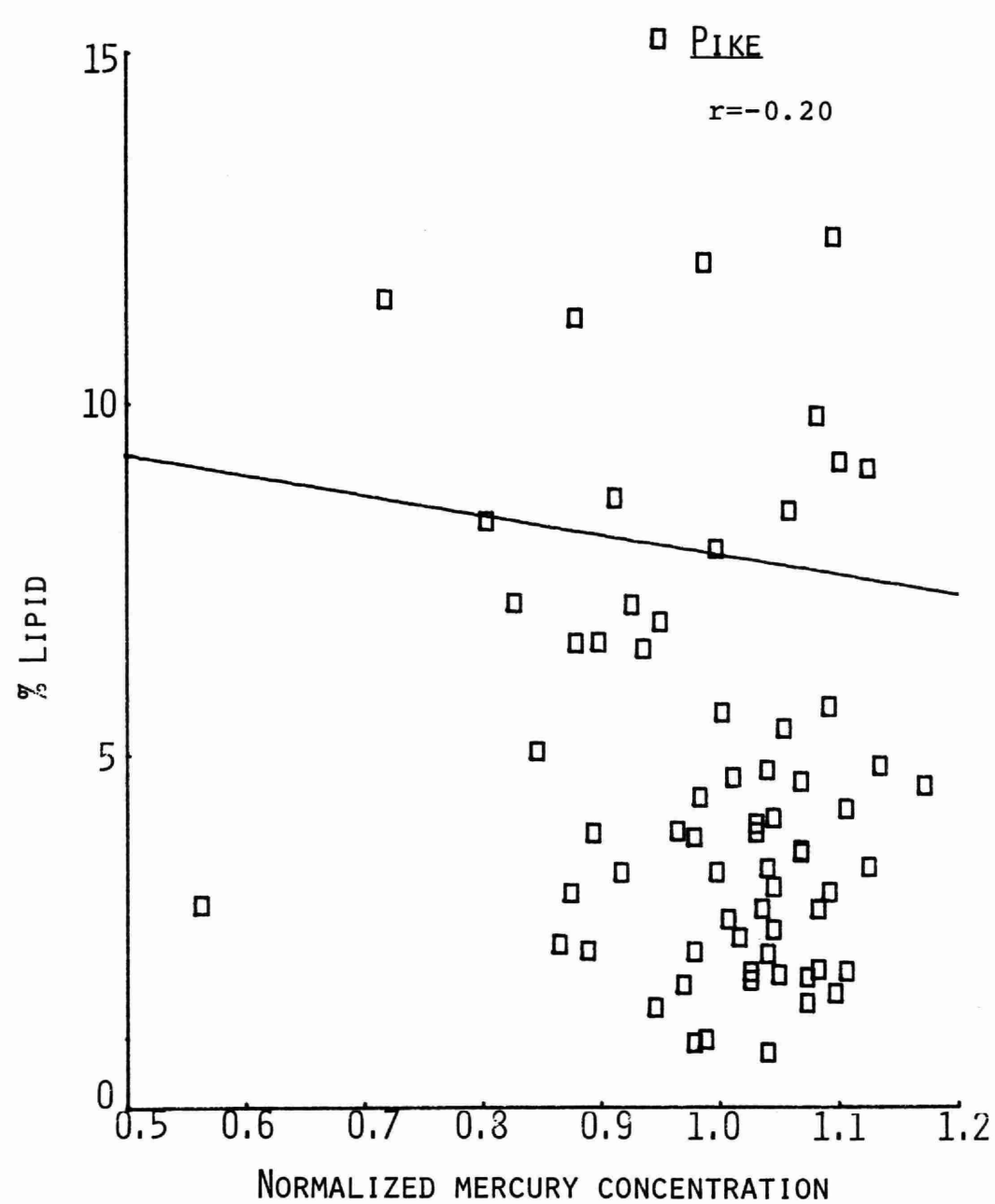
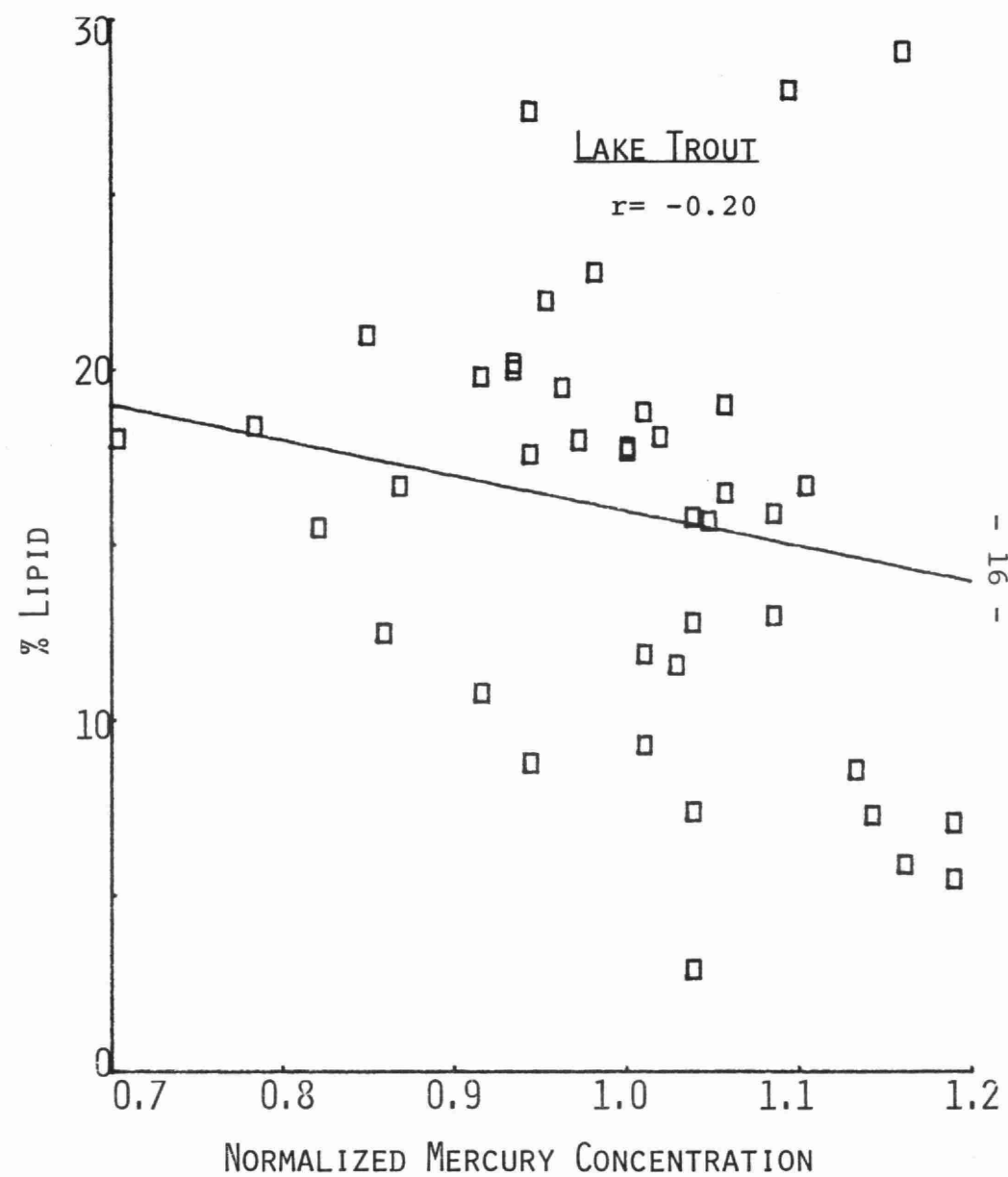
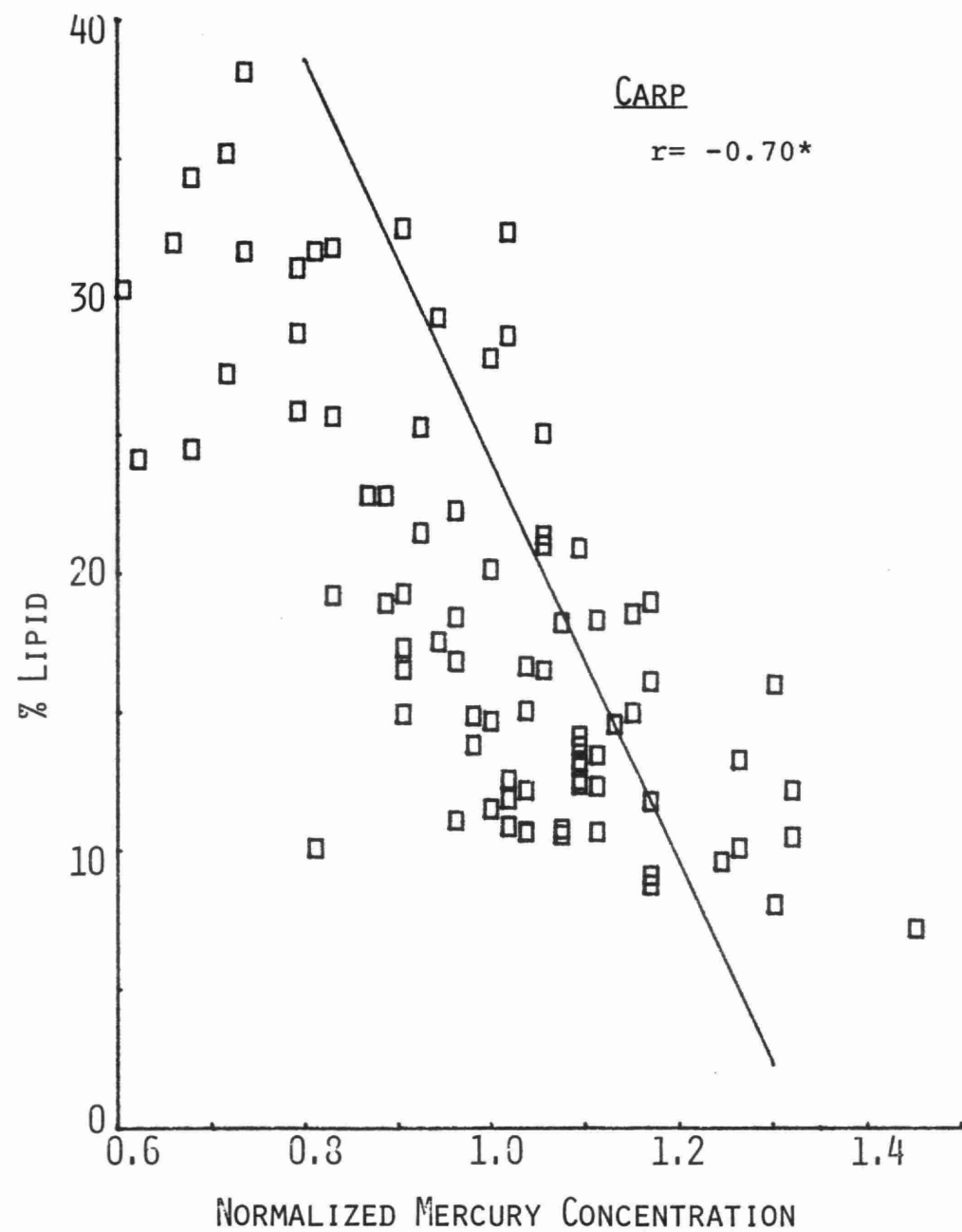
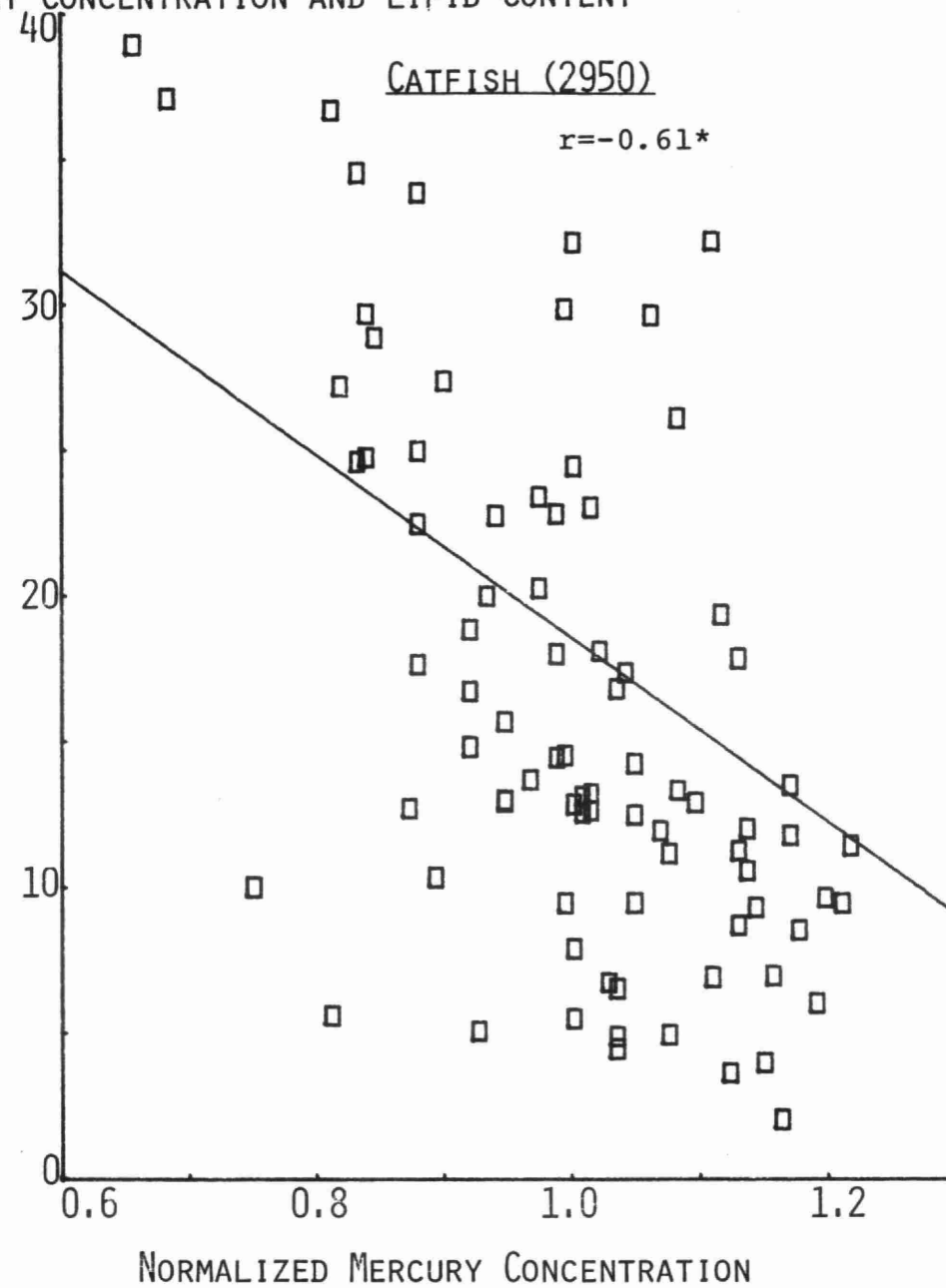
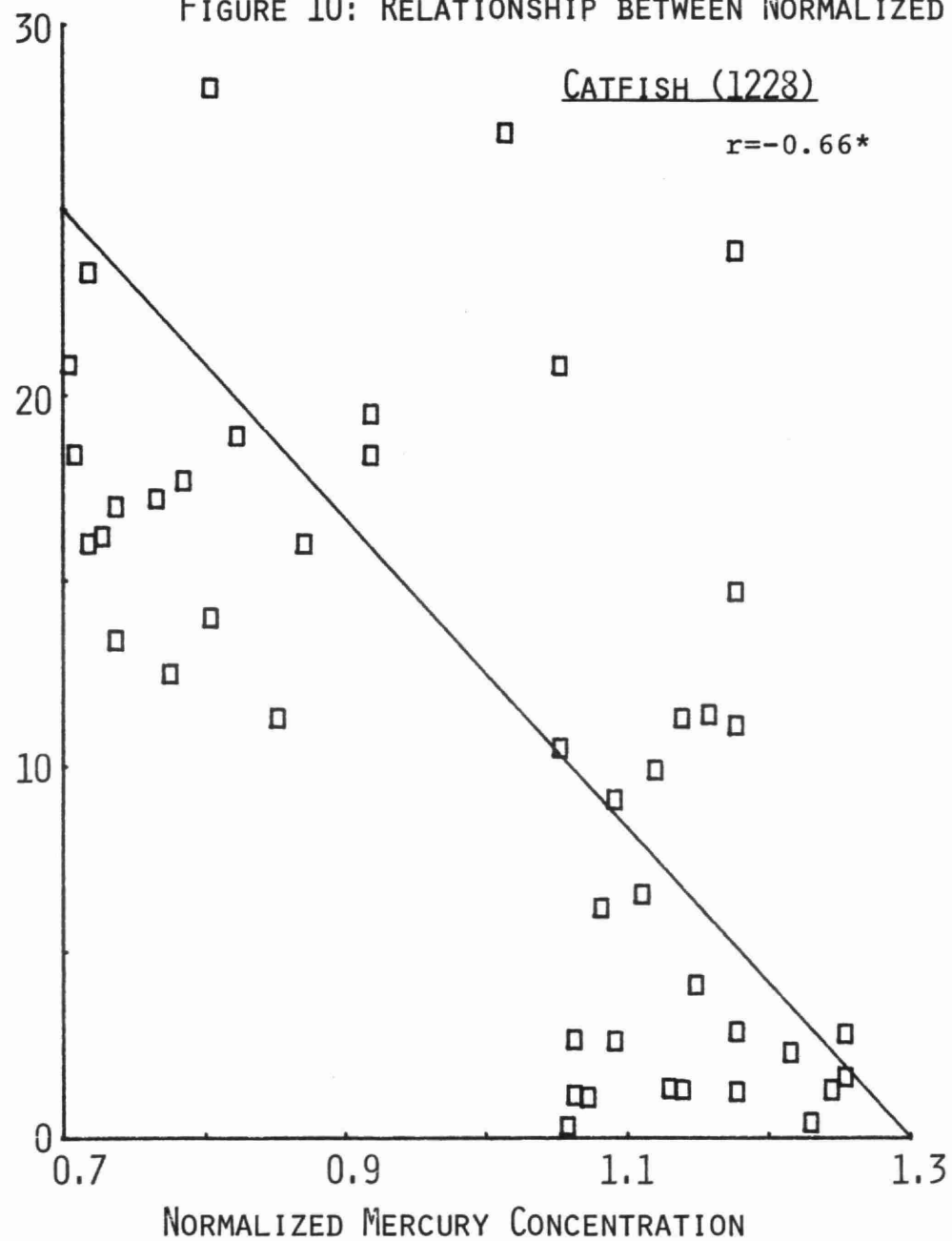


FIGURE 9: RELATIONSHIP BETWEEN NORMALIZED MERCURY CONCENTRATION AND LIPID CONTENT



* SIGNIFICANTLY DIFFERENT FROM ZERO ($p < 0.01$)

FIGURE 10: RELATIONSHIP BETWEEN NORMALIZED MERCURY CONCENTRATION AND LIPID CONTENT



* SIGNIFICANTLY DIFFERENT FROM ZERO ($P < 0.01$)

FIGURE 11:

RELATIONSHIP BETWEEN LIPID AND WATER CONTENT

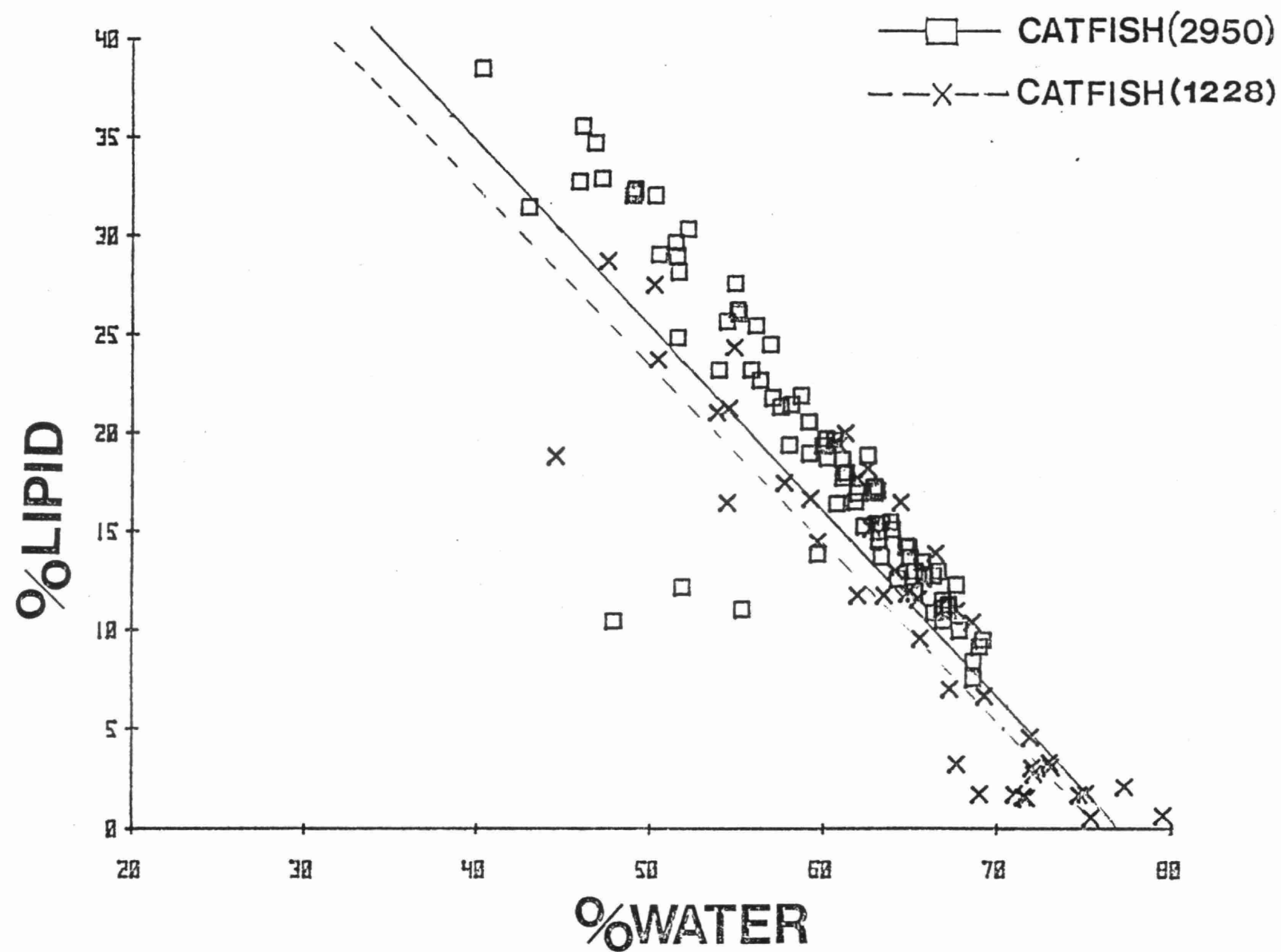
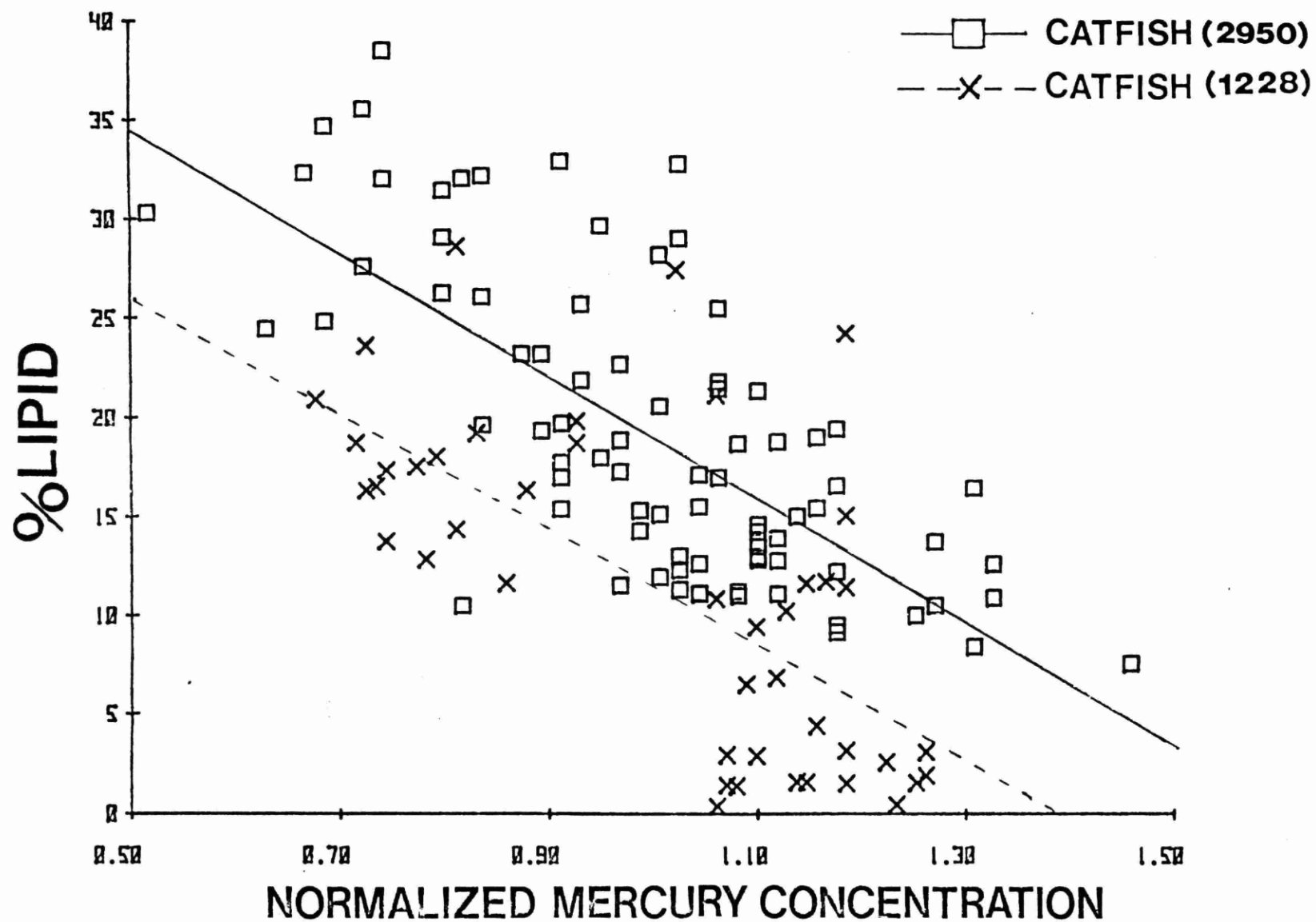


FIGURE 12: RELATIONSHIP BETWEEN NORMALIZED MERCURY CONCENTRATION AND LIPID CONTENT



d) It is interesting to speculate on these relationships, and the lack of them in the pike, walleye, and lake trout. The most obvious difference between the species is in their feeding habits. The pike, pickerel, and lake trout are predaceous fish, while the carp and catfish are omnivorous bottom feeders. The musculature of the former group is quite different from that of the omnivores. The predaceous fish have more dark muscle, evidently used for extended continuous movement. Dark muscle is high in lipid, and uses the lipid as an energy source, but apparently contains the same (5) or higher (4) mercury concentration than the white muscle.

The carp and catfish are fairly sedentary, and their musculature is primarily white. It is in these fish that the mercury-relationship is evident. The high lipid content of the dark muscle in the predaceous fish would suggest a lower mercury content, but this is apparently counteracted by the high metabolic rate in the dark muscle, which is likely to result in an increase in the rate of uptake of mercurials (4). The walleye, the fish with the most dark muscle, even shows evidence of mercury-water and mercury-lipid relationships opposite to those observed in the other fish.

Contrary to what has been reported for organophosphate and chlorinated pesticides, lipid solubility does not appear to be of any importance in the retention of methyl mercury in fish tissue.

D: ORGAN DISTRIBUTION OF MERCURY:

The distribution of mercury, expressed as a ratio between the mercury concentration in the organ and the average muscle concentration, is shown in Figure 13. Our data, along with data from other studies (5, 12) on organ distribution is shown in Figure 14. It can be seen that the organ distribution is quite unique for each of the species. The M.O.E. data agrees quite well (except for pickerel) with other published data.

De Frietas et al (6) has used the organ distribution of mercury as an indicator of the mode of mercury accumulation. He compared the organ ratios of pike from a contaminated lake with the ratios from pike experimentally contaminated with methyl mercury via food and water routes. The ratios observed in the pike from the contaminated lake agreed quite well with those observed in the fish experimentally contaminated via water. The organ ratios derived from our analysis of pike, however, are intermediate between the two sets of experimental ratios. The pike used in this study was from an area with no known industrial input of mercury.

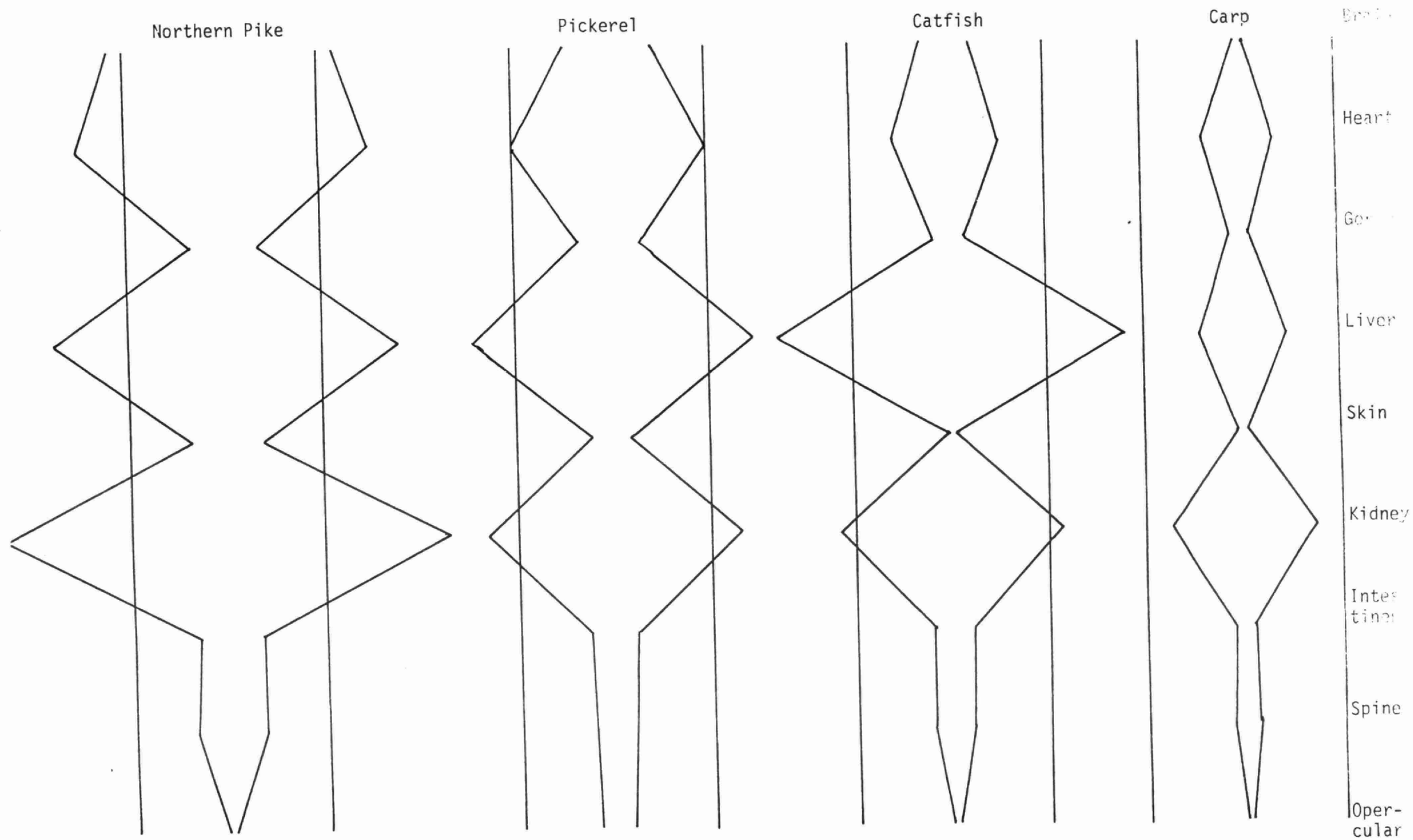


FIGURE 13:
Organ to muscle ratio profiles: MOE data only

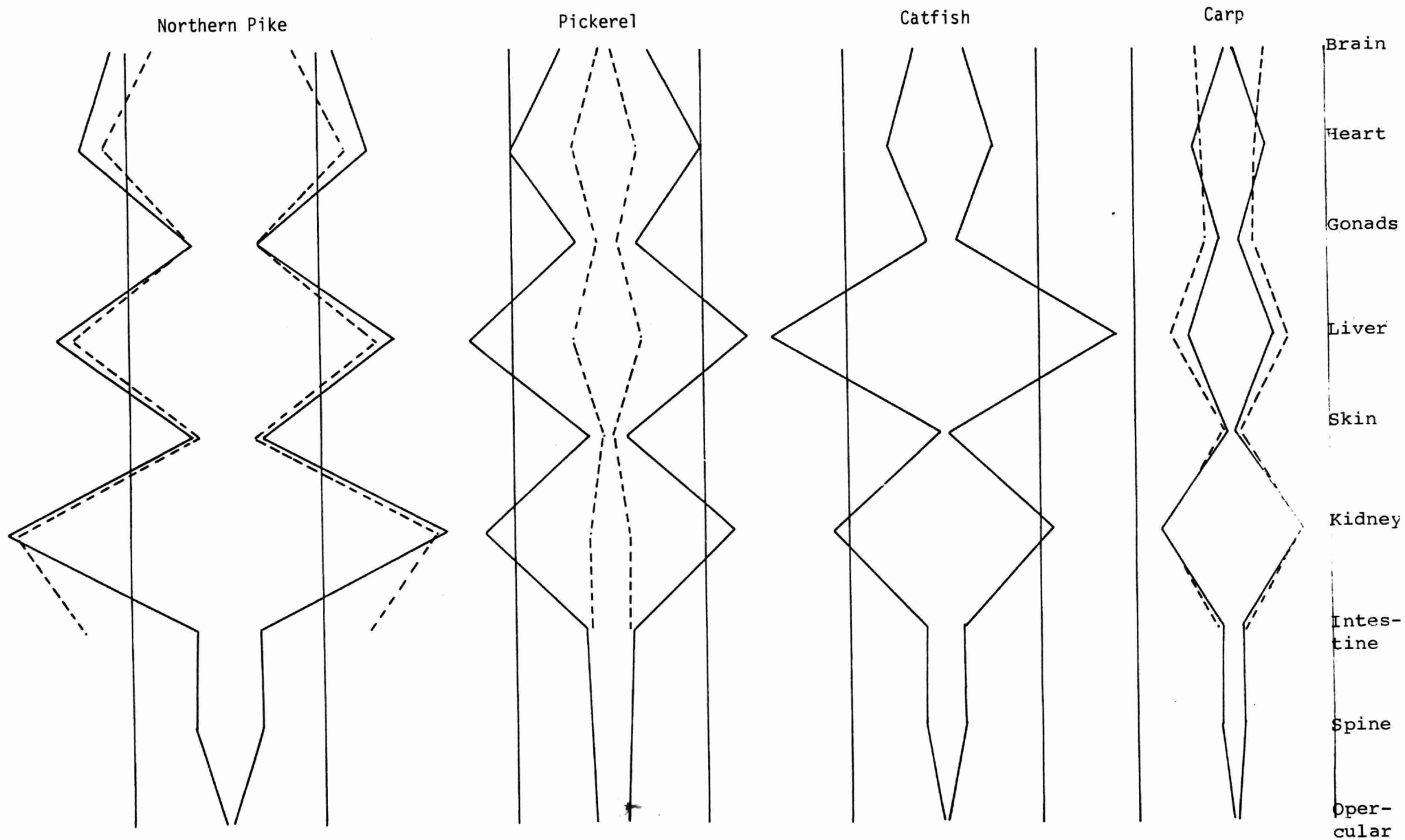


FIGURE 14:
Organ to muscle ratio profiles (MOE and other Data)

E: MISCELLANEOUS CONCLUSIONS:

a) Mercury Balance in Sample Fractions

Several samples of fish muscle were analyzed for total mercury, then water and lipids were removed by azeotropic distillation. The lipids, water, and remaining dried muscle were then analyzed for total mercury, to establish the absolute amount of mercury in each fraction of the sample.

The residual fish muscle retained 99.3%, the lipids held 0.6%, and the water only 0.1% of the total mercury in the sample. This supports the observation that mercury is bound to the proteinaceous material in fish flesh.

b) Methyl Mercury to Total Mercury Ratio

Methyl mercury was determined as well as total mercury on some samples. Fifty muscle samples were analyzed this way for catfish, northern pike, and lake trout. The average methyl/total mercury ratios for these species were 91%, 90%, and 95%, respectively.

The methyl mercury data once again followed the trend of higher results for anterior as opposed to posterior muscle samples. Also the general tendency of higher mercury in the interior sections compared to exterior sections of muscle was observed for the methyl mercury data.

Methyl mercury was determined on some organs for northern pike and lake trout. The following ratios of methyl of total mercury were found:

TABLE 3: METHYL MERCURY IN VARIOUS ORGANS

ORGAN	<u>METHYL Hg/TOTAL Hg RATIO (%)</u>	
	Northern Pike	Lake Trout
Kidney	56	100
Liver	56	55
Brain	56	
Skin	54	95
Scales	58	67
Gonads	58	64
Intestine	55	95
Spine	53	

The values for the Northern pike are remarkably similar to one another, and are much lower than the muscle ratio of 90% observed for this specimen. The data for the Lake Trout is in reasonable agreement with data published by other workers (2,5).

c) Fish Sampling Techniques: "Snip" vs Homogenates

From the conclusions reached regarding the variability in mercury distribution along and throughout some species, it was reasonable to assume that "snip" samples (that is, small samples of flesh snipped from a fillet) would not provide the same precision as would samples obtained by taking a large portion of fillet, blending it, and sub-sampling the homogenate. This was confirmed by comparing the variance on samples taken both ways; the overall variance using duplicate "snips" samples was nearly double that for duplicate homogenate samples.

IV: SUMMARY

The mercury concentrations in muscle samples from the tail of the fish were invariably lower than those from the anterior muscle samples. Mercury concentration is generally higher in interior muscle section, for bottom feeding omnivorous species. For these same species (Channel catfish and carp) there was a negative relationship between lipid content and mercury concentration, and positive relations between mercury and water concentration in muscle. Pike, walleye and lake trout (predaceous fish) showed no such significant relationships.

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The distribution of mercury in various organs of the fish was as follows for most species:

Kidney > liver > heart > brain > gonads > intestines.

Skin, scales, eyes, bone, and other external parts were invariably less in mercury content than muscle from the same fish. Nearly all of the mercury in fish muscle was found to be associated with muscle protein rather than lipids or water fractions, and was virtually all in the methyl form.

Methyl/total ratios as low as 53% were found in some tissues of N. Pike, and 55% for L. Trout. It was concluded that to ensure optimum analytical precision, fish fillets should be homogenized and sub-samples, as opposed to taking "snip" samples.

REFERENCES

- 1) Westöö G., Rydalv M., "Kvicksilver och Metylkvicksilver i Fisk och Kräfter", Var Föda 1969:3
- 2) Suzuki T., et al., "The Chemical Form and Bodily Distribution of Mercury in Marine Fish", Bull. Env. Contam. and Toxicol., 10:347-355 (1973).
- 3) Hannerz L., "Experimental Investigations on the Accumulation of Mercury in Water Organisms", Institute of Freshwater Research, Drottningholm, Report No. 48:119-176 (1968).
- 4) Bäckström J., "Distribution Studies of Mercuric Pesticides in Quail and Some Fresh-Water Fishes", Acta Pharmacol. et Toxicol., 27: Supplementum 3; 1-103 (1969).
- 5) Lockhart W.L., et al., "Methylmercury in Northern Pike (Esox Lucius): Distribution, Elimination, and Some Biochemical Characteristics of Contaminated Fish", J. Fish. Res. Bd. Canada 29:1519-1523 (1972).
- 6) De Frietas A.S.W., et al., "Origins and Fate of Mercury Compounds in Fish", in Proc. Int. Conf. on Transport of Persistent Chemicals in Aquatic Ecosystems, III-31, Ottawa, Canada, May 1-3, (1974).
- 7) Bishop J.N., et al., "The Determination of Mercury in Environmental Samples", Ontario M.O.E. Laboratory Report, (1974).
- 8) Westöö G., "Determination of Methyl mercury Compounds in Foodstuffs", Acta. Chem. Scand. 21: 1790 (1967).
- 9) Personal Communication, O.W. Berg, Ontario M.O.E. Laboratory, (1974).
- 10) Snedecor G. W., "Statistical Methods", Iowa State College Press, Ames, Iowa, (1956).

- 11) Brandes C.H., Dietrich R., "Observations on the Correlations Between Fat and Water Content and the Fat Distribution in Commonly Eaten Fish", Veröff. Inst. Meeresforsch. Bremerh., 5: 299-305, (1958).
- 12) Potter L., et al., "Mercury Levels in Lake Powell", Env. Sci. and Technol., 9: 41-46 (1975).